



**UNIVERSITI PUTRA MALAYSIA**

**EFFECTS OF OIL PALM FROND METHANOLIC EXTRACT ON BLOOD  
PRESSURE, ANTIOXIDANT STATUS AND SELECTED ORGANS OF  
NITRIC OXIDE-DEFICIENT RATS**

**JULIANA BT MD JAFFRI**

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STATUS AND SELECTED ORGANS OF NITRIC  
OXIDE-DEFICIENT RATS**

**By**

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**Thesis submitted to the School of Graduate Studies, University Putra  
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Doctor of Philosophy**

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## DEDICATIONS

This thesis is dedicated to my late mother, who passed on before I completed this study, and who would have been very proud of my achievements.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy.

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Hypertension is a modifiable risk factor for cardiovascular disease (CVD). Diet intervention by increasing the intake of fruits and vegetables proved to be beneficial by reducing blood pressure (BP) and lowering the risk of CVD. Apart from micronutrients, the phytochemical content of fruits and vegetables has been associated with the BP lowering effect. Hypertension is commonly associated with a reduced vascular nitric oxide (NO) due to excessive degradation by reactive oxygen species (ROS). In addition, NO is also important in maintaining organ functions and perfusion. In this study normal and NO deficient rats were used to evaluate the BP lowering effect and antioxidative property of oil palm frond methanolic extract (OPFME). The protective effect of OPFME in the liver, heart, kidney and brain of these rats were assessed and any toxicity effect was also investigated. Two major groups of rats (normal and NO deficient groups) were further divided into three sub-groups; the first subgroup received the vehicle used to dilute OPFME; the

second subgroup received OPFME and the third subgroup received captopril. NO deficiency was induced by administration of N- $\omega$ -nitro-L-arginine methyl ester (L-NAME). Erythrocyte/organ superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx) activities, and plasma/organ malondialdehyde (MDA) concentration were measured. Livers, hearts, kidneys and brains were removed at the end of the study. Histological appearances of these organs were examined. Kidneys were functionally evaluated by measurement of plasma creatinine and urea concentrations, whereas liver function was tested by measuring plasma alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP). OPFME significantly attenuated BP increases in NO deficient rats comparable to captopril, and temporarily improved antioxidant status. In the liver OPFME partially protected hepatocytes from extensive injury due to NO deficiency. OPFME also partially prevented coronary arterioles from thickening, but failed to inhibit structural changes in the left ventricle. The glomeruli of OPFME treated NO deficient rats were not improved. Slight renal tubular dilation observed in normal rats treated with OPFME is a matter of concern and should be further investigated. OPFME showed remarkable hippocampal neuroprotection and this correlated well with its antioxidative property in the brain. There was no neurotoxic effect of OPFME observed. On the contrary, neurogenesis was displayed as an increase in the number of viable granule cells of the dentate gyrus (DG) of normal rats treated with OPFME. In conclusion, OPFME was effective in suppressing BP increment during NO deficiency. The extract was also partially hepatoprotective and significantly neuroprotective. However, OPFME may induce nephrotoxicity and should be investigated further for clarification.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah.

**KESAN EKSTRAK METHANOL DAUN KELAPA SAWIT TERHADAP TEKANAN DARAH,  
TAHAP ANTIOKSIDAN DAN ORGAN TERPILIH PADA TIKUS KEKURANGAN NITRIK  
OKSIDA**

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Hipertensi adalah factor risiko penyakit kardiovaskular yang boleh diatasi. Pengubahsuaian cara pemakanan dengan melebihkan buah-buahan dan sayur-sayuran telah terbukti dapat mengurangkan tekanan darah serta mengurangkan risiko penyakit kardiovaskular. Selain dari zat-zat makanan asas, kandungan polifenol buah-buahan dan sayur-sayuran juga dikaitkan dengan penurunan tekanan darah. Hipertensi adalah penyakit yang sering dikaitkan dengan penurunan kandungan nitrik oksida vaskular akibat tindakbalas radikal oksigen yang tidak terkawal. Nitrik oksida juga adalah penting untuk fungsi dan pengaliran darah ke organ-organ badan. Kajian ini dijalankan untuk menilai kesan ekstrak daun kelapa sawit terhadap penurunan tekanan darah dan tahap antioksidan badan. Kesan-kesan terhadap hati, ginjal, jantung dan otak juga dikaji untuk menentukan tahap perlindungan dari kegagalan yang berpunca dari kekurangan nitrik oksida. Selain daripada itu pemerhatian terhadap kesan-kesan toksik ekstrak daun kelapa sawit

juga dijalankan. Tikus digunakan untuk kajian ini, dan telah dibahagikan kepada dua kumpulan utama iaitu normal dan kekurangan nitrik oksida. Setiap kumpulan dibahagikan kepada tiga kumpulan kecil mengikut rawatan yang diberikan. Kumpulan pertama menerima pelarut yang digunakan untuk melarutkan ekstrak, kumpulan kedua menerima ekstrak dan kumpulan ketiga menerima captopril. Kesan kekurangan nitrik oksida dihasilkan daripada pemberian N- $\omega$ -nitro-L-arginine methyl ester (L-NAME). Tahap aktiviti superoxide dismutase (SOD), catalase dan glutathione peroxidase (GPx) dalam sel darah merah/organ serta kandungan malondialdehyde plasma/organ dikaji. Organ-organ yang dipilih untuk dikaji adalah hati, ginjal, jantung dan otak. Kajian histologi dilakukan ke atas organ-organ tersebut. Tahap creatinine plasma dan urea dilakukan untuk menentukan fungsi ginjal, manakala kandungan alanine aminotransferase (ALT), aspartate aminotransferase (AST), dan alkaline phosphatase (ALP) dilakukan untuk menentukan fungsi hati. Ekstrak daun kelapa sawit didapati berkesan mengekalkan tekanan darah normal. Tahap antioksidan juga bertambah baik dengan pengambilan ekstrak walaupun tidak kekal hingga ke akhir tempoh kajian. Ekstrak tersebut juga didapati meredakan kerosakan sel hati yang teruk akibat kekurangan nitrik oksida, serta mengurangkan (tidak ketara) penebalan salur darah koronari tetapi tidak berkesan merencat perubahan struktur pada ventrikel kiri jantung. Tiada kesan perlindungan diperhatikan di glomerulus. Ekstrak didapati menyebabkan sedikit pembengkakan pada tiub ginjal, dan ianya perlu dikaji dengan lebih lanjut. Kesan ekstrak pada sel-sel otak di hippocampus adalah sangat baik di mana ianya mungkin disebabkan oleh peningkatan tahap antioksidan otak. Tiada kesan sampingan berlaku di organ ini akibat rawatan ekstrak, malah di bahagian dentate

gyrus sel-sel neuron didapati bertambah bilangannya. Kesimpulannya, ekstrak daun kelapa sawit berkesan mengawal peningkatan tekanan darah semasa di dalam keadaan kekurangan nitrik oksida. Ekstrak juga mengurangkan kegagalan hati (tidak ketara), serta dapat melindungi dari kerosakan sel-sel otak. Walau bagaimana pun ekstrak mungkin boleh menyebabkan kesan toksik di ginjal. Oleh itu, kajian lanjut perlu dijalankan untuk menjelaskan masalah ini.



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## DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.



**JULIANA BT MD JAFFRI**

Date: 28<sup>th</sup> November 2008

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## LIST OF ABBREVIATIONS

$\cdot\text{O}_2^-$	Superoxide
$\cdot\text{OH}^-$	Hydroxyl radical
ACE	Angiotensin converting enzyme
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
ANOVA	Analysis of variance
ARB	Angiotensin II receptor blocker
AST	Aspartate aminotransferase
AT <sub>1</sub>	Angiotensin II Type 1
BHT	Butylated hydroxytoluene
BP	Blood pressure
BSA	Bovine serum albumin
BUN	Blood urea nitrogen
CA	Cornu Ammonis
cAMP	Cyclic adenosine monophosphate
cGMP	Cyclic guanosine monophosphate
CNS	Central nervous system
Cu/ZnSOD	Copper and zinc superoxide dismutase
CVD	Cardiovascular disease
DASH	Dietary approach to stop hypertension
DG	Dentate gyrus
DTNB	dithio-bis(2-nitrobenzoic acid)

EC-SOD	Extracellular superoxide dismutase
EDHF	Endothelium derived hyperpolarizing factor
EDTA	Ethylene diamine triacetic acid
eNOS	Endothelial nitric oxide synthase
GAE	Gallic acid equivalent
GPx	Glutathione peroxidase
GSH	Reduced glutathione
GTP	Guanosine triphosphate
H&E	Hematoxylin-eosin
HSI	Hepatosomatic index
iNOS	Inducible nitric oxide synthase
L-NAME	N- $\omega$ -nitro-L-arginine methyl ester
LSEC	Liver sinusoidal endothelial cell
LVH	Left ventricular hypertrophy
MDA	Malondialdehyde
MnSOD	Manganese superoxide dismutase
NADH	Nicotinamide adenine dinucleotide, reduced form
NADPH	Nicotinamide adenine dinucleotide phosphate, reduced form
NF- $\kappa$ B	Nuclear factor kappa B
NMDA	N-methyl-D-aspartate
nNOS	Neuronal nitric oxide synthase
NOS	Nitric oxide synthase
OPFME	Oil palm frond methanolic extract

PGE <sub>2</sub>	Prostaglandin E <sub>2</sub>
PUFA	Polyunsaturated fatty acid
RAS	Renin angiotensin system
ROS	Reactive oxygen species
RWP	Red wine polyphenols
SDS	Sodium dodecyl sulfate
SOD	Superoxide dismutase
TBA	Thiobarbituric acid
TEP	1,1,3,3-tetraethoxypropane
TPC	Total phenolic content
UPM	Universiti Putra Malaysia
VSM	Vascular smooth muscle
WKY	Wistar Kyoto

# CHAPTER 1

## INTRODUCTION

Hypertension is a lifestyle related disease which can be devastating if complicated. It is a common risk factor for CVD (Schnall et al, 2000; Grundy et al, 1999), affecting millions of people throughout the world and in 2006 about 43% of the adult Malaysian population (30 years and above) have diastolic blood pressure of 90 mmHg or greater, a relative increase of 30% from that of 10 years earlier (Ministry of Health, 2008).

There is still much uncertainty about the pathophysiology of hypertension. Decreased level of NO, a very important mediator of vasorelaxation, is a well known factor for increased blood pressure (Forte et al, 1997). This condition is also associated with obesity/insulin resistance, whereby obese/insulin-resistant subjects are characterized by endothelial dysfunction and endothelial resistance to insulin's effect on enhancement of endothelium-dependent vasodilation (Steinberg et al, 1996). The low NO level could contribute to the development of hypertension. Epidemiological studies indicate that two major determinants of human morbidity and mortality, arterial hypertension and insulin resistance, are related, suggesting the possibility of a common underlying mechanism (Duplain et al, 2001; Modan et al, 1985; Lucas et al, 1985).



CVD development is a multi-factorial process in which endothelial dysfunction, inflammatory response, dyslipidemia, and activated platelets all play significant role in the process (Hansson, 2005; Libby and Simon, 2001). The seriousness of the Malaysian situation has prompted The Ministry of Health to launch a number of campaigns in an effort to prevent CVD from becoming widespread. As the rising number of individuals with hypertension are identified and treated, the incremental burden on the Malaysian government healthcare system could be staggering.

A nutritional approach to this problem would be especially appealing, because it could minimize both the healthcare workload and the cost of therapy. In addition, nutritional approach rarely results in unwanted side effects, which is very common with most pharmacological therapy. Based on the Dietary Approach to Stop Hypertension (DASH) diet (Most et al, 2004), the intake of plant foods high in phytochemicals is associated with lowering of BP. An alternative way of increasing phytochemical intake is through supplementation with plant based nutraceuticals, in a convenient capsule/tablet form that is compact and easy to take. Individuals with strong family history of obesity and metabolic syndrome may benefit from this approach, as the problem is usually dealt with at an early stage, whereby it is still uncomplicated and reversal of the condition is possible. Oil palm frond extract may be a good candidate for a nutraceutical in this category, which may help reduce the risk of developing hypertension in individuals with low endothelial NO.

The majority of nutraceuticals in Malaysia is imported from countries such as USA and Australia, utilizing plants from the temperate climate. This is one of the reasons